Clinical Study Report

A Phase I/IIa sporozoite challenge study to assess the protective efficacy of new malaria vaccine candidates AdCh63 AMA1, MVA AMA1, AdCh63 MSP1, MVA MSP1, AdCh63 ME-TRAP & MVA ME-TRAP

VAC039

REC Reference: 10/H0505/30
EudraCT number: 2010-018341-56
CTA number: 44802/101071/19/407

CONFIDENTIAL

CONTENTS

1. DECLARATION

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Signed:  Date: 15/3/2012

Print name: Professor A.V.S. Hill

Chief Investigator

Signed:  Date: 15/03/2012

Print name: Dr S.H. Sheehy

Lead Clinician & Report Author

Signed:  Date: 15/03/2012

Print name: Dr A.M. Lawrie

Head of Regulatory Affairs & Senior Project Manager

2. OVERVIEW

Study title:	A Phase I/IIa sporozoite challenge study to assess the protective efficacy of new malaria vaccine candidates AdCh63 AMA1, MVA AMA1, AdCh63 MSP1, MVA MSP1, AdCh63 ME-TRAP & MVA ME-TRAP
Trial code:	VAC039
Study description:	Open label observational challenge study.
Test IMPs:	AdCh63 MSP1, MVA MSP1, AdCh63 AMA1, MVA AMA1, AdCh63 ME-TRAP, MVA ME-TRAP
Indication studied:	Safety, immunogenicity and efficacy
Sponsor:	University of Oxford
Chief Investigator:	Professor Adrian V.S. Hill; Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford, UK
Co-Investigators:	Dr Saul N. Faust; Wellcome Trust Clinical Research Facility University of Southampton, UK Dr Tom Doherty; University College London Clinical Research Facility, London, UK
Study centres:	Centre for Clinical Vaccinology and Tropical Medicine Churchill Hospital Old Road Headington Oxford OX3 7LJ Wellcome Trust Clinical Research Facility Southampton General Hospital C Level, West Wing Mailpoint 218 Tremona Road SO16 6YD University College London Clinical Research Facility c/o Rayne Building 5 University Street London WC1E 6JJ Infection and Immunity Section Sir Alexander Fleming Building

	Imperial College of Science, Technology and Medicine Imperial College Road London SW7 2AZ
Clinical Phase:	I/IIa
Study dates planned:	1st June 2010 – 31st December 2011
Study dates actual:	12th July 2010 – 15th March 2011
Enrolment:	Completed
Publication:	In preparation
GCP Statement:	This study was performed in compliance with ICH Good Clinical Practice (GCP) including the archiving of essential documents.

3. PROTOCOL SYNOPSIS

Objectives	<u>Primary Objective:</u> To assess if volunteers who receive the novel vaccine candidates; AdCh63 MSP1, MVA MSP1, AdCh63 AMA1, MVA AMA1, AdCh63 ME-TRAP and MVA ME-TRAP in heterologous prime boost regimens are protected wholly or partially against malaria infection in a sporozoite challenge model. This will be determined by noting the number of subjects who develop malaria infection and the time in hours between exposure and parasitaemia as detected by thick-film blood smear compared with controls. <u>Secondary Objectives:</u> (i) To assess the safety of the immunisation regimens alone and during co-administration. (ii) To assess immunogenicity of the vaccine regimes by measuring T cell responses (IFN-γ ELISPOT, flow cytometry) and antibody responses (ELISA, B cell assays, GIA) to MSP1, AMA1 and ME-TRAP antigens before and after malaria infection. If there is evidence of partial or complete protection, we will explore immunological correlates of protective immunity. <u>Tertiary Objective:</u> To assess long term protective efficacy of AdCh63 MSP1, MVA MSP1, AdCh63 AMA1, MVA AMA1, AdCh63 ME-TRAP and MVA ME-TRAP in heterologous prime boost regimens by re-challenging any volunteers protected at initial malaria challenge.
Trial design	Non randomised, un-blinded Phase I/IIa trial in healthy, malaria-naïve adults.
Sample Size	<u>Group 1</u> 8-10 volunteers: 1 dose of AdCh63 MSP1 5 x 10^{10} vp intramuscularly and 1 dose MVA MSP1 2 x 10^{8} pfu intramuscularly 8 weeks later (range 6-12 weeks) followed by sporozoite challenge 12-28 days later. <u>Group 2</u> 8-10 volunteers: 1 dose of AdCh63 AMA1 5 x 10^{10} vp intramuscularly and 1 dose MVA AMA1 1.25 x 10^{8} pfu intramuscularly 8 weeks later (range 6-12 weeks) followed by sporozoite challenge 12-28 days later. <u>Group 3</u> 8-10 volunteers: 1 dose of AdCh63 AMA1 5 x 10^{10} vp intramuscularly and 1 dose AdCh63 MSP1 5 x 10^{10} vp intramuscularly co-administered into separate arms followed 8 weeks later (range 6-12 weeks) by 1 dose of MVA AMA1 1.25 x 10^{8} pfu intramuscularly and 1 dose MVA MSP1 2 x 10^{8} pfu

	intramuscularly co-administered into separate arms (but the same arm as the corresponding AdCh63 vaccine) followed by sporozoite challenge 12-28 days later. <u>Group 4</u> 8-10 volunteers: 1 dose of AdCh63 MSP1 5 x 10^{10} vp intramuscularly and 1 dose AdCh63 ME-TRAP 5 x 10^{10} vp intramuscularly co-administered into separate arms followed 8 weeks later (range 6-12 weeks) by 1 dose of MVA MSP1 2 x 10^{8} pfu intramuscularly and 1 dose MVA ME-TRAP 2 x 10^{8} pfu intramuscularly co-administered into separate arms (but the same arm as the corresponding AdCh63 vaccine) followed by sporozoite challenge 12-28 days later. <u>Group 5</u> 6 non-vaccinated controls for sporozoite challenge Total: 38-52 volunteers
Main criteria for inclusion	Volunteers must satisfy all the following criteria to be eligible for the study: • Healthy adults aged 18 to 50 years. • Able and willing (in the Investigator's opinion) to comply with all study requirements. • Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner. • For female volunteers, willingness to practice continuous effective contraception for the duration of the study. • Agreement to refrain from blood donation during the course of the study. • Written informed consent.
Duration of treatment	All volunteers in groups 1-4 received one or two vaccines at enrolment, one or two vaccines 8 weeks later and sporozoite challenge 12-28 days later (experimental malaria infection). Six volunteers underwent sporozoite challenge only.
Criteria for Evaluation of Objectives	<u>Primary Objective:</u> The number of vaccinees who develop malaria infection and the time between exposure and parasitaemia as detected by thick-film blood smear, compared with controls. <u>Secondary Objective:</u> • Analysis of actively and passively collected data on adverse events from diary cards, clinical review of volunteers and laboratory measurements.

	• Immunological assays of cellular and humoral immunity. <u>Tertiary Objective:</u> To assess long term protective efficacy of AdCh63 AMA1, AdCh63 MSP1, AdCh63 ME-TRAP, MVA AMA1, MVA MSP1 and MVA ME-TRAP in heterologous prime boost regimens by re-challenging any volunteers protected at initial malaria challenge.
Statistical methods	Kaplan Meier analysis of efficacy data. Descriptive analysis of safety and immunology data.
Blinding	Non-Blinded
Controls	Controlled
Randomisation	Non Randomised

4. ETHICS AND REGULATORY APPROVAL

INDEPENDENT ETHICS COMMITTEE APPROVAL

The study protocol and related documents were reviewed and approved by Berkshire Research Ethics Committee. The initial ethical approval for the trial was given on 7th May 2010, and where appropriate, all subsequent substantial amendments were approved by this committee prior to implementation.

ETHICAL CONDUCT OF THE STUDY

The study was performed in accordance with the declaration of Helsinki and in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

VOLUNTEER INFORMATION & CONSENT

The volunteer information sheet detailed the procedures involved in the study (aims, methodology, potential risks and anticipated benefits) and the Investigator explained these verbally to each volunteer prior to obtaining consent. The volunteer then signed and dated the informed consent form to indicate that they fully understood the information, and were willing to participate in the study. Volunteers were given copies of the signed consent form to keep for their records. The original consent forms are kept in a confidential file in the Investigators' records. All volunteers provided written informed consent to participate in the study prior to being screened.

REGULATORY APPROVAL

The study was performed in compliance with the requirements of the Medicines and Healthcare products Regulatory Agency (MHRA);

- CTA number: 44802/101071/19/407
- EudraCT number: 2010-018341-56

The study was approved by the MHRA on 27th April 2010. Where appropriate, all subsequent substantial amendments were submitted to the MHRA for approval prior to implementation.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Title	Name and affiliation
Chief Investigator & Principal Investigator - Oxford	Professor A.V.S. Hill – University of Oxford
Principal Investigator	Dr S.N. Faust – Wellcome Trust Clinical Research Facility, University of Southampton
Principal Investigator	Dr T Doherty – University College London Clinical Research Facility, London
Trial Clinicians	Dr S Sheehy – University of Oxford Dr C Duncan – University of Oxford Dr N Anagnostou – University of Oxford Dr T Havelock – Wellcome Trust Clinical Research Facility, University of Southampton D T Mahungu – University College London Clinical Research Facility, London
Project Managers	Dr A Lawrie – University of Oxford Dr K Gantlett – University of Oxford
Monitor	Ms S Saunders –Appledown Monitoring Ltd Ms C Dobson – Appledown Monitoring Ltd
Laboratory Investigators	Dr S Draper – University of Oxford Dr S Biswas – University of Oxford Mr S Elias – University of Oxford Mr P Choudhary – University of Oxford Mr N Edwards – University of Oxford

6. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

AMA1 Insert
AMA1 polymorphism presents a potential problem for the development of a widely effective vaccine. Most allelic diversity is reported in domains I and III in response to immune selection pressure. Studies of naturally exposed individuals have shown both strain-specific and cross-reactive antibody responses, but only responses against the entire ectodomain, rather than individual epitopes correlate with protection. In this trial we attempt to address the problem of polymorphism of AMA1, at least in part, by including two divergent alleles of *Plasmodium falciparum* AMA1 (3D7 and FVO) in tandem as the vaccine insert in both AdCh63 and MVA.

MSP1 Insert
The insert encodes a composite sequence from the blood-stage *P. falciparum* malaria antigen MSP1. To generate vectored vaccine candidates suitable for clinical assessment in the challenging area of blood-stage vaccine development we included i) the four N-terminal conserved regions (Blocks 1, 3, 5 & 12) to generate T cell responses to more conserved rather than very variable regions of MSP1; ii) two allelic variants of the C-terminus of MSP1 ($MSP1_{42}$) arrayed in tandem in the vectored insert; and iii) recently described point mutations in $MSP1_{19}$, to enhance overall immunogenicity and increase the likelihood of developing protective "inhibitory antibodies" rather than unwanted "blocking" antibodies.

ME-TRAP Insert
ME-TRAP contains a fusion protein of multiple epitopes (ME) and the *P. falciparum* pre-erythrocytic thrombospondin-related adhesion protein (TRAP). The 'ME' is a string of 20 epitopes, mainly CD8 T cell epitopes from *P. falciparum* pre-erythrocytic antigens, fused to TRAP. The individual CTL epitopes which constitute the 'multiple epitope' part of ME-TRAP represent a variety (six) of potentially protective target antigens and are included to ensure an immune response to the vaccine in the majority of the population vaccinated. The ME string is fused to the entire sequence of the T9/96 strain of *P. falciparum* TRAP and the ME-TRAP hybrid is a 2398 base-pair insert which encodes for a single polypeptide of 789 amino acids. TRAP was selected as it is well characterized abundant pre-erythrocytic stage antigen and has a protective homologue in rodents.

AdCh63 MSP1, AdCh63 AMA1 & AdCh63 ME-TRAP were all manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility (CBF) in Oxford where final certification and associated labelling also took place. Further details relating to batch release and manufacturing of these investigational products can be found in the relevant IMP-Ds.

MVA MSP1, MVA AMA1 & MVA ME-TRAP were all manufactured under Good Manufacturing Practice conditions by Impfstoffwerk Dessau-Tornau (IDT), Germany. Final certification of these products and associated labelling took place at the Clinical Biomanufacturing Facility (CBF) in Oxford. Further details relating to batch release and manufacturing of these investigational products can be found in the relevant IMP-Ds.

The vials of all vaccines were stored between –70°C and –90°C, in a locked freezer, at either the Clinical Biomanufacturing Facility, the Centre for Clinical Vaccinology & Tropical Medicine or

Wellcome Trust Clinical Research Facility, University of Southampton. All movements of the study vaccines between sites or from locked freezers to clinic rooms were fully documented.

7. STUDY POPULATION

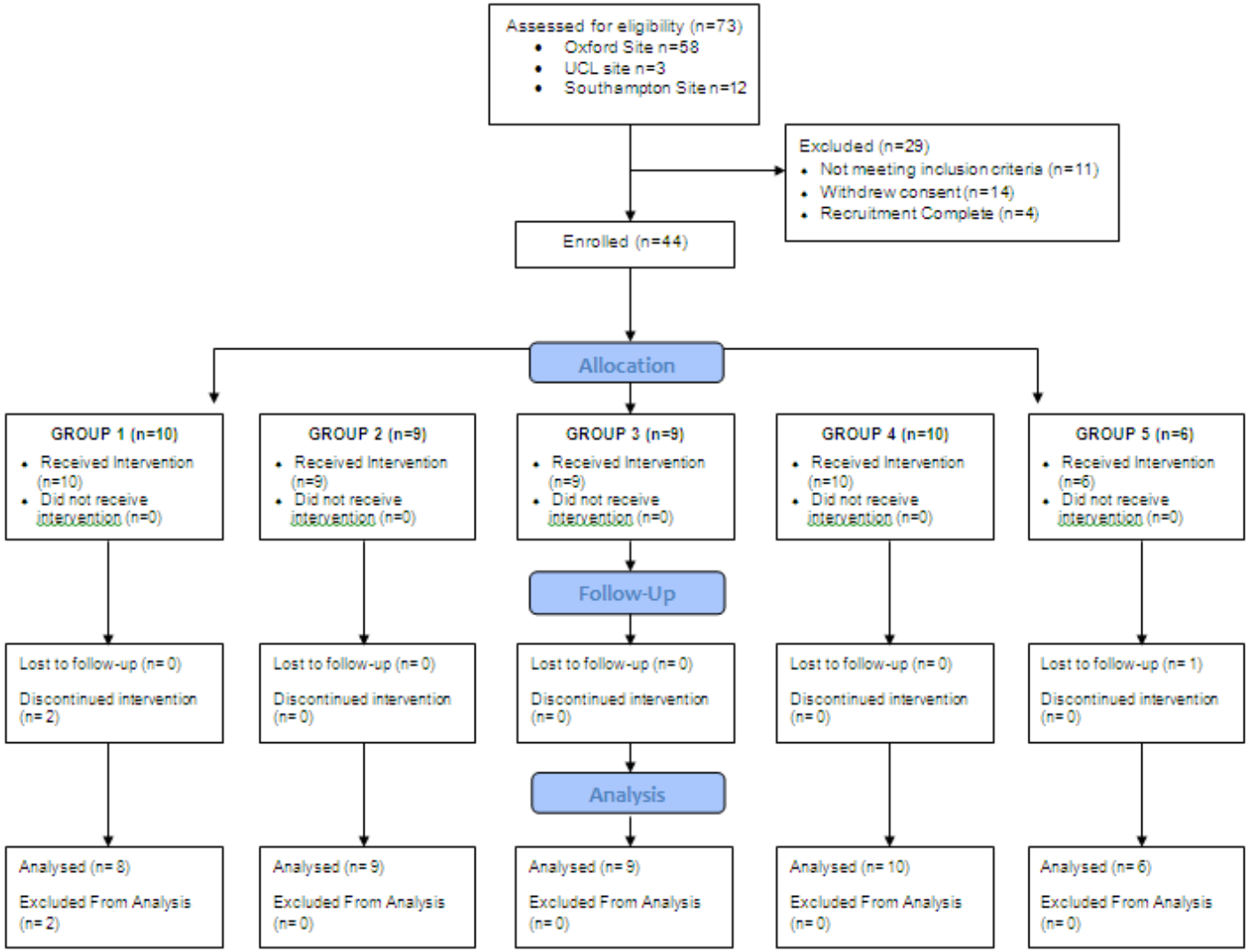

Figure 1: CONSORT diagram of study progress. "Analysis" refers to analysis of primary objective; Vaccine efficacy

3 volunteers were withdrawn from the trial:

- **M1039 805** – Group 1 – Withdrawn at Day 66 due to SAE unrelated to study intervention (see below).
- **M2039 907** – Group 1 – Withdrawn 6 days post challenge (C+6) due to SAE unrelated to study intervention (see below).
- **M1039 840** – Group 5 – Withdrawn following visit on day 90 post challenge as volunteer moved abroad.

8. PROTOCOL DEVIATIONS

Site at Which Deviation Occurred	*Oxford*	*London*	*Southampton*
Entry criteria	0	0	0
Withdrawal criteria	0	0	0
Incorrect dosing regimen	6*	0	0
Concomitant medication	0	0	0
Other**	9	0	2
Total	15	0	0

Table 1: Protocol deviations

**'Incorrect Dosing Regimen' included the following Protocol Deviations;*

- The total dose of AdCh63 ME-TRAP administered to volunteer M1039 834 was 4.59 x 10^{10} instead of the 5 x 10^{10}vp specified in the protocol. This was because vial number 70 of batch 02 of AdCh63 ME-TRAP was under filled giving a reduced extractable volume.
- The dosing of Riamet in 4 individuals deviated from that specified in the protocol due to volunteer error. There were no safety implications as a result of these deviations.
- 1 individual was wrongly treated with Riamet when an alternative anti-malarial therapy should have been prescribed.

***'Other' included;*

- 7 interventions or clinical reviews taking place outside the time window specified in the protocol.
- 1 review taking place by telephone rather than in clinic.
- C+150 visit did not take place for 1 volunteer
- 1 volunteer received an unnecessary additional needle puncture.
- 1 volunteer visited a malaria endemic region during the follow-up period post challenge.

9. RESULTS

9.1 DEMOGRAPHICS OF STUDY POPULATION

Volunteer group	Mean age at Screening (range)	Gender (% Male)
1 (n=10)	27.2 (21-38)	30%
2 (n=9)	28.6 (19-39)	44%
3 (n=9)	31.2 (21-48)	56%
4 (n=10)	27.2 (19-40)	50%
5 (n=6)	35.2 (21-50)	67%

Table 2: Demographics of volunteers.

9.2 ADVERSE EVENTS

(a) Serious Adverse Events (SAEs)

Four SAEs occurred during the study;

M1039 805 – Female – Group 1 – Recruited at Oxford Site
10 days post MVA MSP1 this volunteer was admitted to hospital for surgical treatment of appendicitis. This was deemed by investigators and the local safety monitor to be unlikely to be related to study vaccinations. The volunteer was withdrawn from the study at this point and did not undergo malaria challenge.

M1039 840 – Male – Group 5 – Recruited at Oxford Site
1 day post malaria diagnosis this volunteer was admitted for in-patient management of malaria symptoms. He was discharged the next day with no long term sequelae. This was a foreseeable AE related to *P. falciparum* infection.

M2039 907 – Male – Group 1 – Recruited at Southampton Site
This volunteer underwent sporozoite challenge on 1st October 2010. He failed to attend his next scheduled study visit on 7th October 2010 when he was formally withdrawn from the study. On 7th October a SAE form was completed with regard to his disappearance. This event was

subsequently felt not to constitute a SAE, and instead was reported immediately to the Medicines and Healthcare products Regulatory Agency (MHRA) as a serious breach of protocol. The MHRA subsequently indicated that this event did not constitute a serious breach of protocol. All other relevant authorities were informed of the event immediately including the sponsor, local REC and local R&D services. It subsequently became clear that from 2nd October 2010 M2039 907 experienced an apparent deterioration in his psychiatric state following a stressful event unrelated to AdCh63-MVA MSP1, sporozoite challenge or any other study related procedure. This event involved police arrest on the evening of challenge for an unrelated previous offence leading to his disappearance for 17 days, a police search and eventual identification of the volunteer in the Netherlands on day 18 post-challenge. He did not have clinical symptoms of malaria on initial review but then developed a fever and was found to be parasitaemic. He was then successfully treated for malaria in the Netherlands. At the time apparent memory loss and suicidal ideation was found resulting in psychiatric in-patient care. He was followed up until it was established that he was under appropriate and responsible clinical care. Consequently, it became clear that M2039 907 has a history of psychiatric morbidity pre-dating his involvement in the study which was not disclosed at screening by the volunteer or his GP. The event was extensively discussed with all investigators and appropriate authorities and non-study related causality was unanimously agreed. It was decided that the deterioration in M2039 907's psychiatric state would be documented formally as a SAE as it resulted in hospitalisation and life threatening illness.

M1039 817 – Female – Group 3 – Recruited at Oxford Site
140 days post sporozoite challenge this volunteer was admitted to hospital with severe lower back pain secondary to spinal stenosis. She had been having episodic back pain for the preceding 30 years. She was discharged 2 days later and referred for out-patient neurosurgical review. This SAE was deemed unlikely to be related to vaccination or sporozoite challenge.

(b) Adverse Events Related to AdCh63 vectored vaccines

The majority of local adverse events (AEs) related to AdCh63 vectored vaccines in this study were mild in severity and all resolved (Figure 2). The AE profile was similar for each vaccine and to AEs seen in other studies using these vaccines.

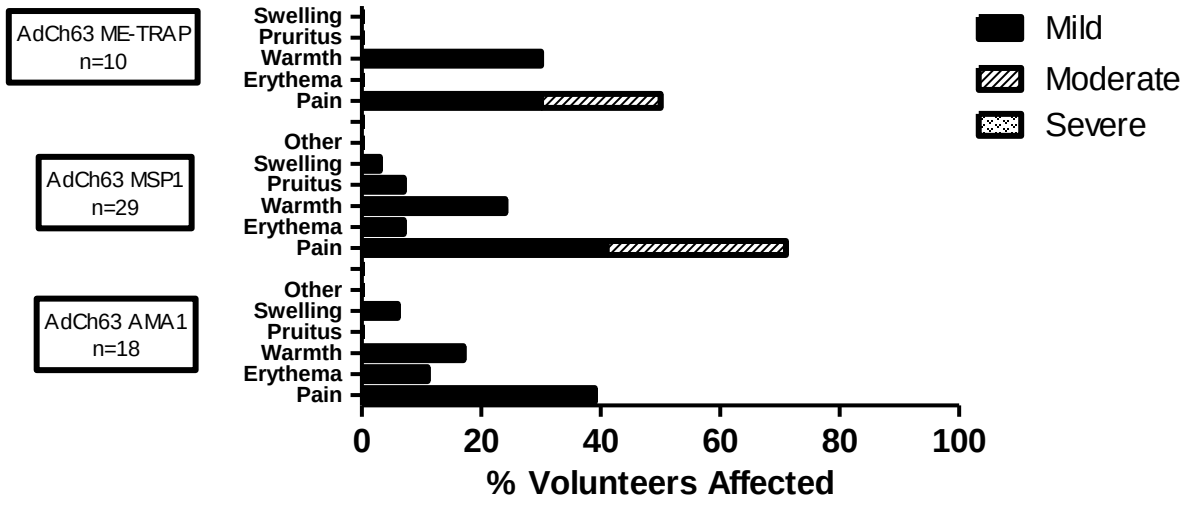

Figure 2: Local Adverse events deemed possibly, probably or definitely related to AdCh63 MSP1 AdCh63 AMA1 and AdCh63 ME-TRAP. The highest intensity adverse event per subject is listed.

Systemic AES following AdCh63 AMA1 and MSP1 administered alone were similar to those seen in previous Phase 1 studies, with the majority of AEs mild in severity (Figure 3). Co-administration of AdCh63 vectored vaccines was systemically more reactogenic than single administration of each individual AdCh63 vectored vaccine, consistent with the increased total dose of AdCh63 administered (Figure 3). AdCh63 MSP1 + AdCh63 AMA1 was a more systemically reactogenic combination than AdCh63 MSP1 + AdCh63 ME-TRAP, despite the total dose of AdCh63 being the same for each group.

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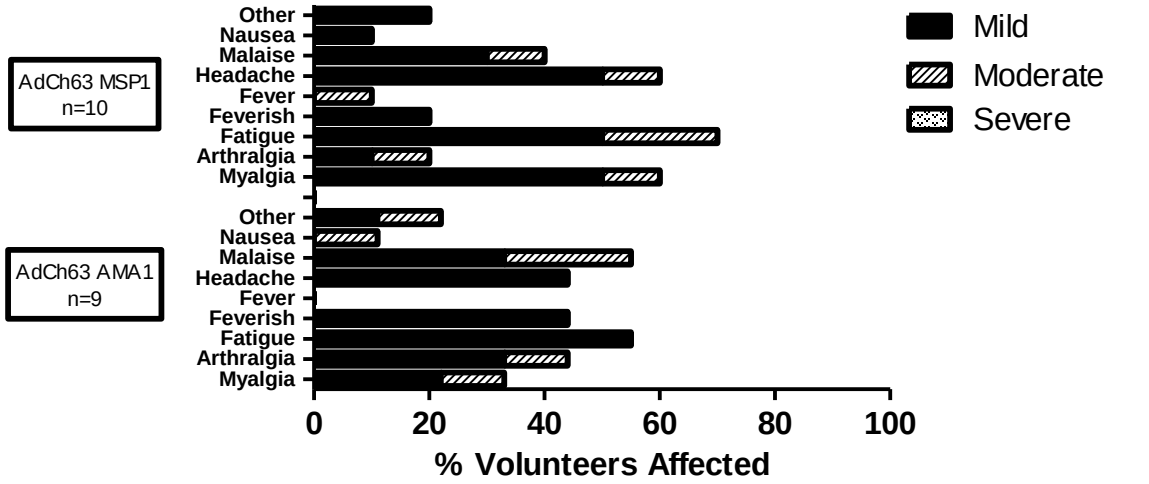

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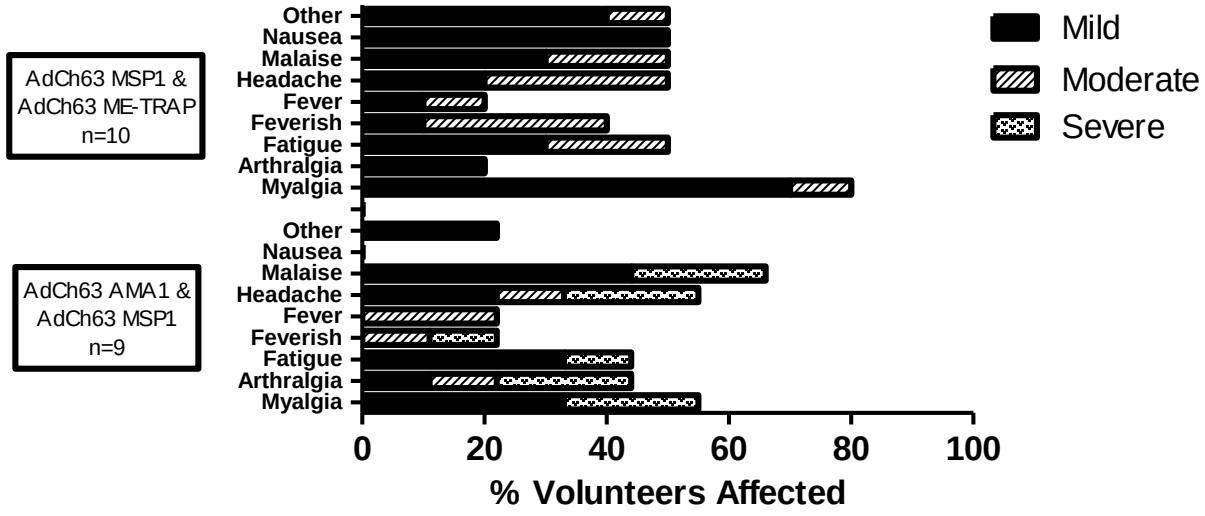

Figure 3: Systemic AEs deemed possibly, probably or definitely related to AdCh63 vectored vaccines. The highest intensity adverse event per subject is listed. Figure 3a: Systemic AEs post AdCh63 MSP1 and AdCh63 AMA1. 'Other' systemic AEs post AdCh63 MSP1 were mild dizziness and low back pain. 'Other' systemic AEs post AdCh63 AMA1 were moderate abdominal cramps and mild left arm tingling. Figure 3b Systemic AEs post co-administration of AdCh63 vectored vaccines. 'Other' systemic AEs post AdCh63 MSP1 + AdCh63 ME-TRAP were moderate dizziness and mild exacerbation of pre-existing psoriasis, mild abdominal pain, mild dizziness and mild loss of appetite. 'Other' systemic AEs post AdCh63 MSP1 + AdCh63 AMA1 included mild loss of appetite and mild tender cervical lymphadenopathy.

(c) Adverse Events Related to MVA vectored vaccines

The majority of local AEs post MVA vectored vaccines in this study were mild in severity and all resolved (Figure 4). The AE profile was similar for each vaccine and to the AE profile seen in other studies using these vaccines.

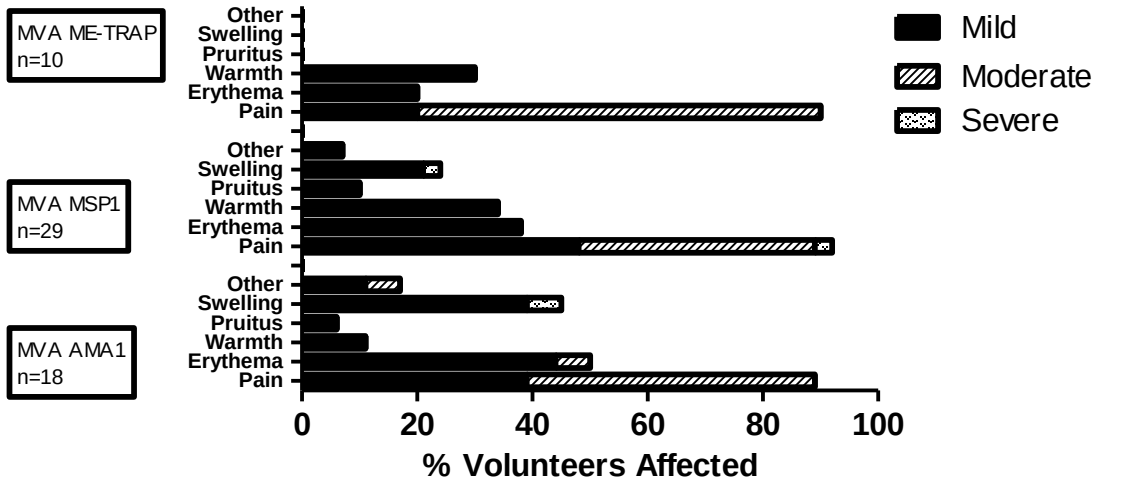

Figure 4: Local AEs deemed possibly, probably or definitely related to MVA MSP1, MVA AMA1 and MVA ME-TRAP. The highest intensity AE per subject is listed. 'Other' local AEs post MVA MSP1 was mild induration. 'Other' local AEs post MVA AMA1 were moderate bruising at vaccination site, mild induration and mildly tender axilla.

The majority of systemic AES following MVA AMA1 and MVA MSP1 administered alone were mild in severity (Figure 5). Co-administration of MVA vectored vaccines was systemically more reactogenic than single administration of each individual MVA vectored vaccine, consistent with the increased total dose of MVA administered (Figure 5). MVA MSP1 + MVA ME-TRAP (total dose of MVA 4 x 10^8 pfu) appeared a slightly more systemically reactogenic combination than MVA MSP1 + MVA AMA1 (total dose of MVA 3.25 x 10^8 pfu) consistent with the increased dose of MVA vector administered in this vaccine combination.

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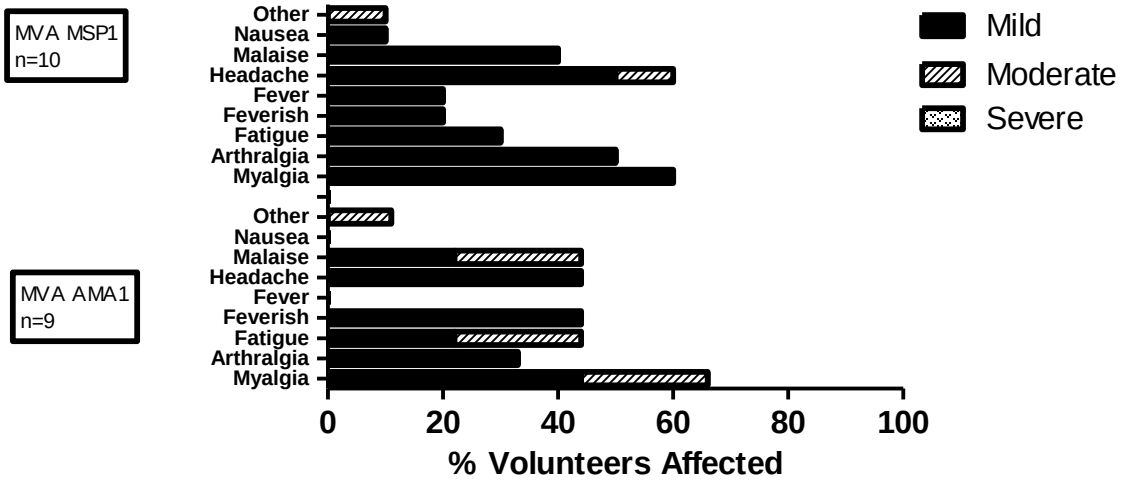

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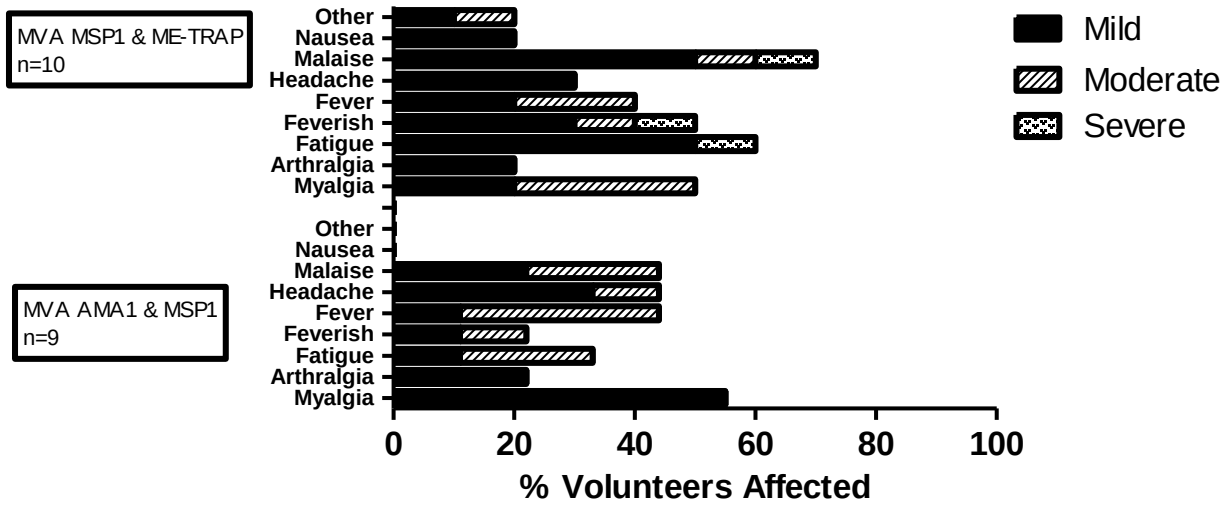

Figure 5: Systemic AEs deemed possibly, probably or definitely related to MVA vectored vaccines. The highest intensity AE per subject is listed. Figure 5a: Systemic AEs post single administration of MVA MSP1 and MVA AMA1. 'Other' systemic AEs post MVA MSP1 was moderate diarrhoea. 'Other' systemic AEs post MVA AMA1 was moderate coryzal symptoms. Figure 5b: Systemic AEs related to co-administration of MVA vectored vaccines. 'Other' systemic AEs post MVA MSP1 + MVA ME-TRAP were mild rhinitis & moderate light-headedness.

(c) Sporozoite Challenge

All vaccinees (groups 1-4) and six un-vaccinated infectivity control volunteers (group 5) underwent sporozoite challenge by the bite of mosquitos infected with the 3D7 strain of *P. falciparum* with the exception of volunteer M1039 805 who was withdrawn from the study prior to challenge (see above). Volunteer M2039 907 was withdrawn from the study 6 days post challenge and the data for this volunteer are not included in the challenge analysis.

No unexpected adverse events or clinical signs of immunopathology were observed in vaccinees post challenge. There was no difference between vaccinees and controls in the duration individuals were symptomatic prior to diagnosis (*P*=0.868) or the number of symptoms present at time of diagnosis (*P*=0.196) (Figure 6).

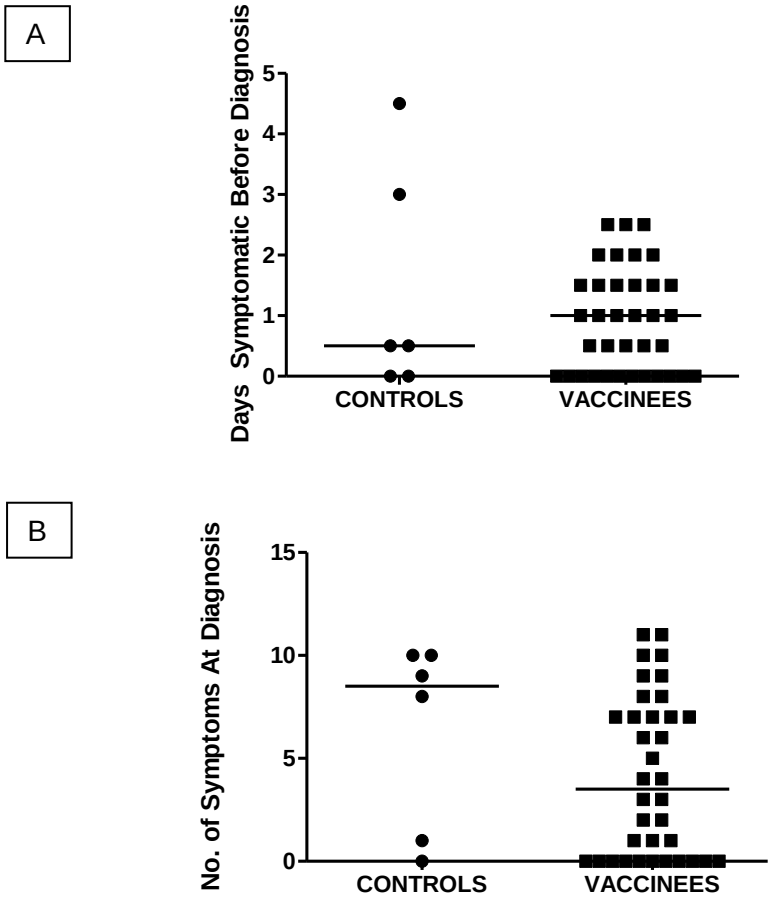

Figure 6: Controls = unvaccinated challenged volunteers (n=6). Vaccinees = Vaccinees who underwent challenge (n=36). Median values for each group are indicated. Figure 6A: No. of days each volunteer demonstrated any clinical symptoms consistent with clinical malaria prior to diagnosis (P=0.868). Figure 6B: No. of symptoms consistent with clinical malaria present on day of diagnosis (P=0.196).

Six of the 41 volunteers (15%) diagnosed with malaria post challenge experienced no symptoms of malaria infection (Figure 7). 16 volunteers (39%) experienced at least one AE post challenge that was severe in severity. Duration of symptoms in volunteers with symptomatic malaria infection ranged from 1-18.5 days (median 4.8 days). There was no difference in duration of symptoms following challenge between vaccinees and controls (*P=0.665*) (Figure 7).

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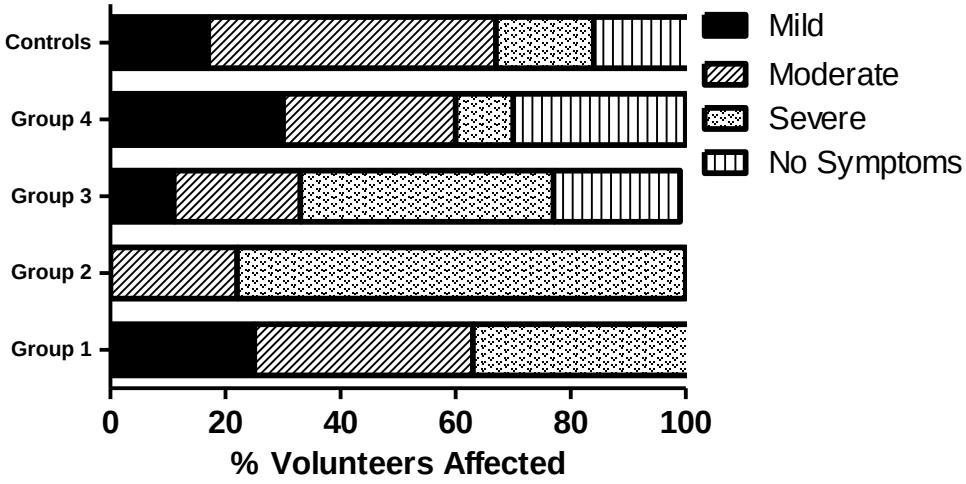

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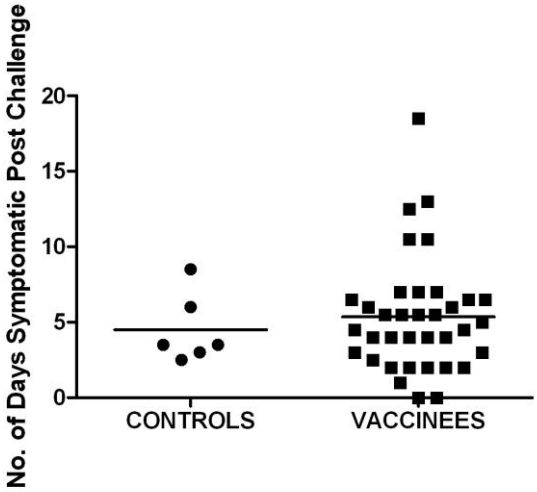

Figure 7: Controls = unvaccinated challenged volunteers (n=6). Vaccinees = Vaccinated volunteers who underwent challenge and were diagnosed with malaria (n=35). Figure 7A: Maximum severity of any symptom post challenge. Figure 7B: No. of days symptomatic post challenge (p=0.58). Median values for each group are indicated.

9.3 CLINICAL LABORATORY EVALUATION

No laboratory AEs were noted that were possibly, probably or definitely related to vaccination. Laboratory AEs deemed unlikely or not related to study interventions are included in Figure 8. Following challenge, all volunteers had safety bloods (including haematological and biochemical analyses) at day 9, 35 and 90 post challenge and within 24 hours of diagnosis with malaria. Leucopenia, lymphopenia, anaemia, neutropenia, elevated alanine aminotransferase (ALT) and thrombocytopenia were seen in some individuals at frequencies and severities expected following *P. falciparum* infection (Figure 9). In addition, 4 volunteers also developed a transient rise in ALT, a known possible complication of Riamet therapy (Table 3).

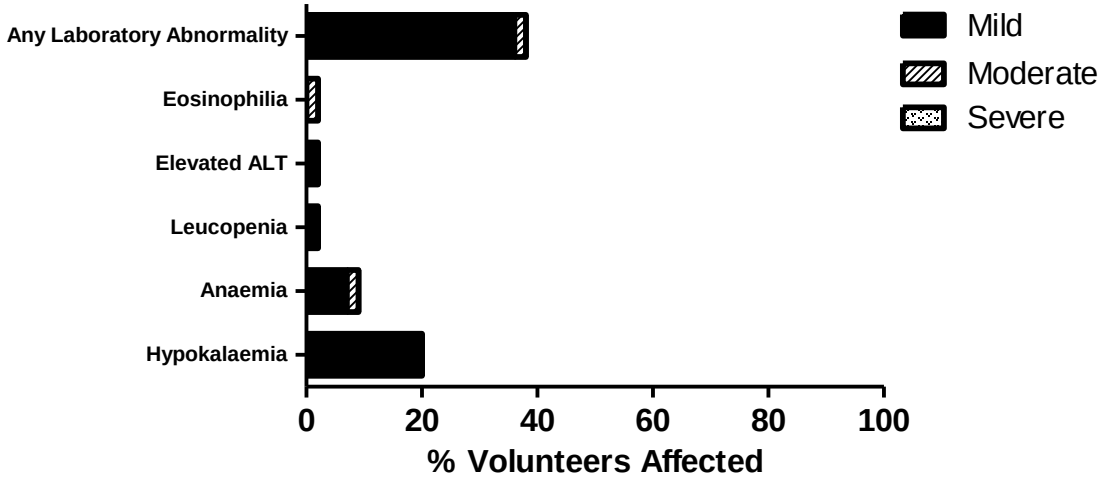

Figure 8: Laboratory AEs occurring in the trial deemed unlikely or not related to study interventions.

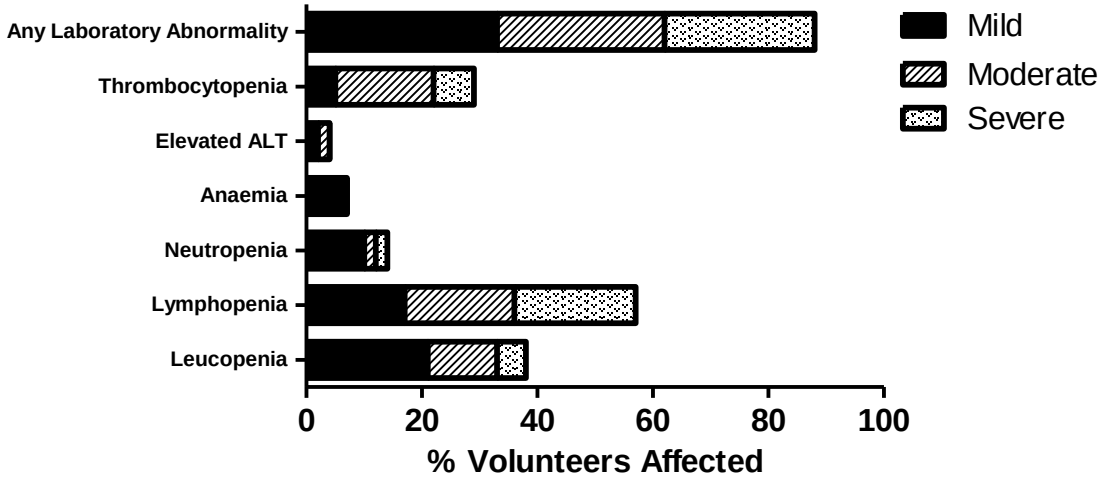

Figure 9: Laboratory AEs post challenge deemed possibly, probably or definitely related to P. falciparum infection. For 'any laboratory abnormality' only the highest intensity AE per subject is counted.

Laboratory Abnormality	Severity	Duration (days)
Elevated ALT	Grade 3	27
Elevated ALT	Grade 2	24
Elevated ALT	Grade 2	14
Elevated ALT	Grade 1	20

Table 3: Laboratory abnormalities post challenge possibly, probably or definitely related to Riamet

9.4 OTHER CLINICAL FINDINGS

There were no adverse events related to study interventions on-going at the end of the study.

9.5 IMMUNOLOGY

Cellular Immune Responses Post Vaccination

Vaccination with AdCh63-MVA induced T cell responses in all volunteers as measured by *ex-vivo* IFN-γ ELIspot (Figure 10). The median ELIspot response in group 1 is, to our knowledge, the highest yet reported following immunization with any subunit vaccine. Co-administration of vaccines was associated with a reduction in the total T cell responses to each individual antigen when compared to single administration (Figure 10B).

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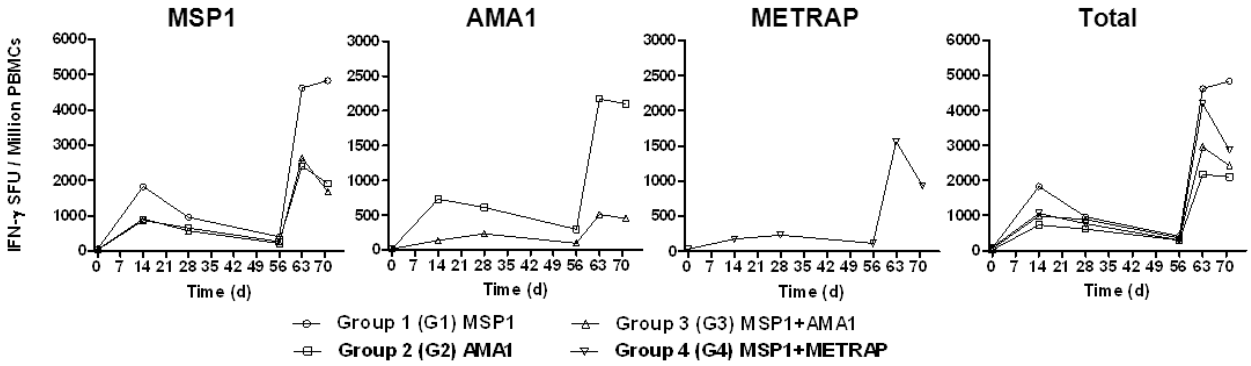

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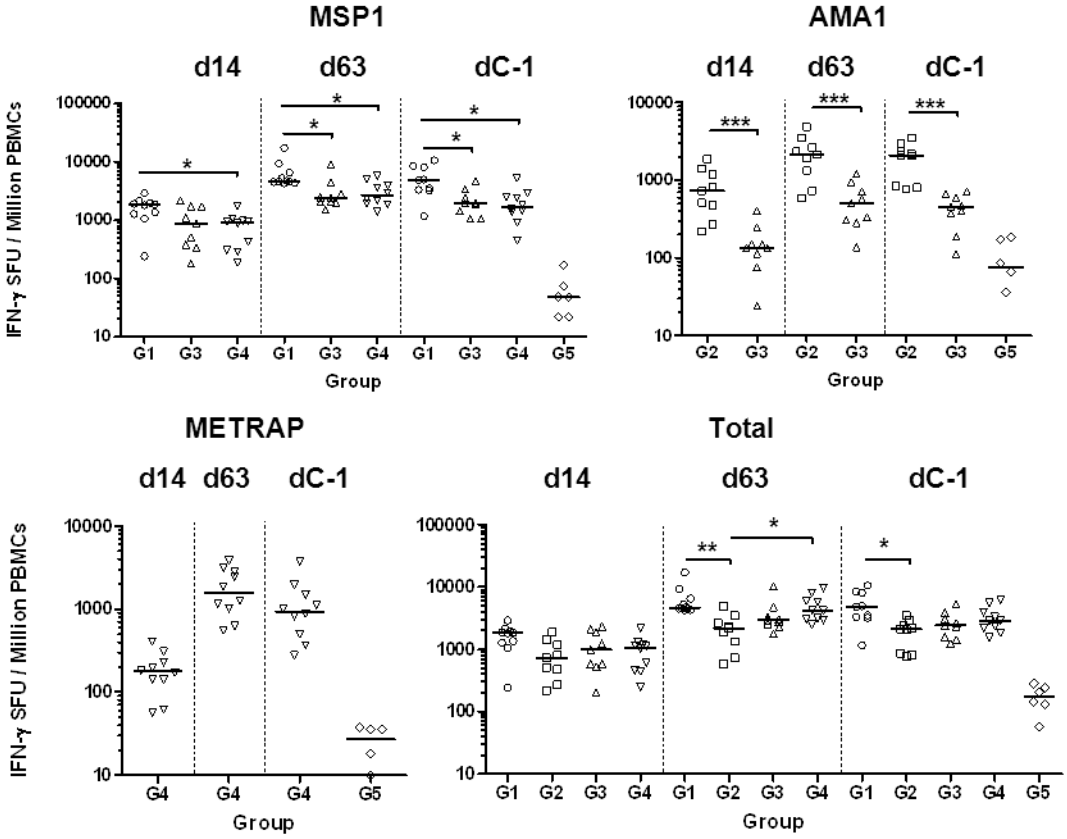
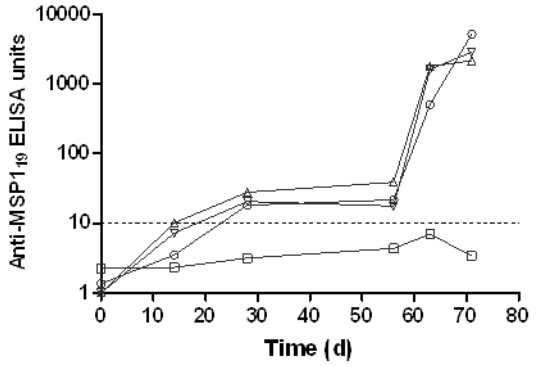


Figure 10: Cellular immunogenicity of ChAd63-MVA immunization regimes.
*Figure 10A: Median ex-vivo IFN-γ ELISPOT responses (summed response across all the individual peptide pools) in PBMC are shown for each relevant Group to the MSP1, AMA1, METRAP antigens. The Total response (summed response to transgene inserts for Groups 3 and 4) is also shown. Figure 10B: Individual and median IFN-γ ELISPOT responses are shown for each antigen and each relevant Group at the day 14, day 63 and dC-1 time-points. * P < 0.05, ** P < 0.01, *** P < 0.001 by Mann-Whitney test (AMA1) and Kruskal-Wallis test with Dunn's Multiple Comparison test (MSP1 and Total). Group 5 was excluded from the dC-1 analysis.*

Humoral Immune Responses Post Vaccination

AdCh63 vaccines primed an IgG antibody response against the target antigen in all volunteers that was boosted considerably by MVA vectored vaccines (Figure 11). Individual vaccine administration induced geometric mean total IgG responses that were highly comparable to those seen in Phase Ia studies, where 40-60µg/ml antigen specific IgG was induced to $MSP1_{19}$ and AMA1 following single vaccine administration. AMA1 IgG responses were significantly reduced when this vaccine was co-administered with the MSP1 vaccines, whereas on average MSP1 responses were maintained (Figure 11).

A



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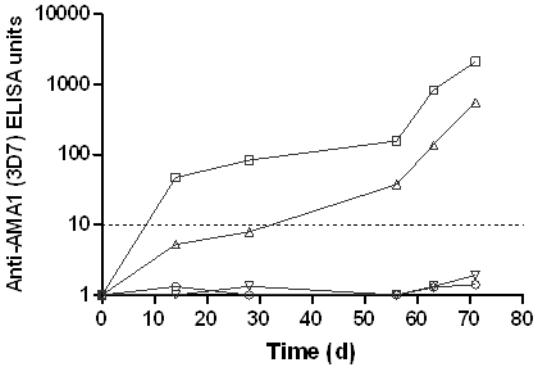
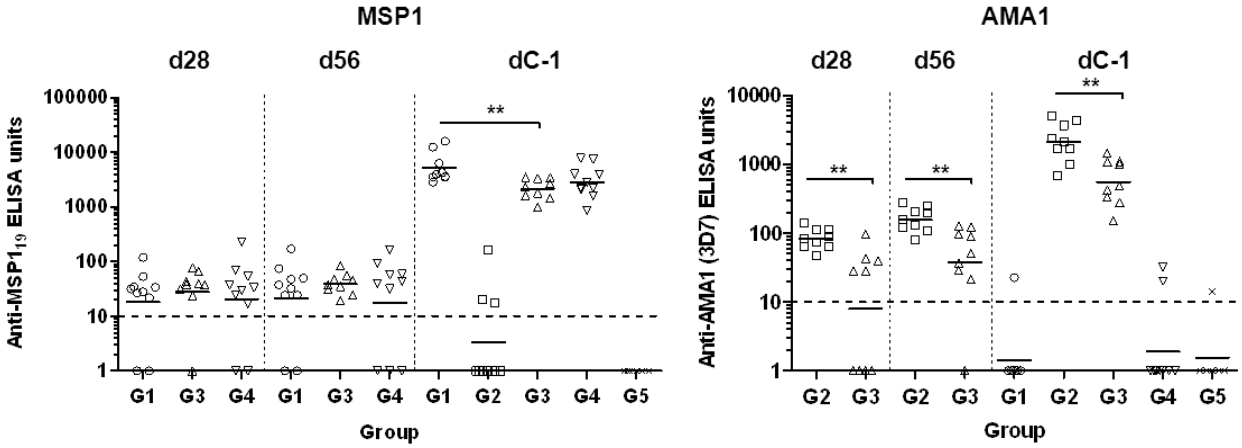


Group 1 (G1) MSP1
Group 2 (G2) AMA1
Group 3 (G3) MSP1+AMA1
Group 4 (G4) MSP1+METRAP

C



D

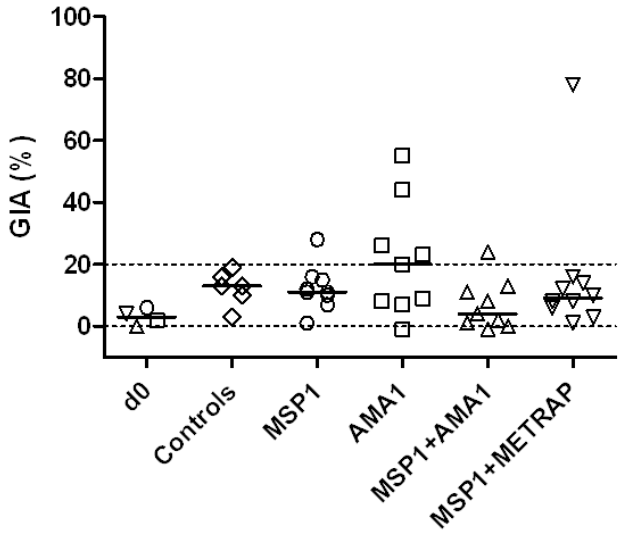


*Figure 11: IgG antibody responses and functional GIA induced by ChAd63-MVA immunization regimes. Geomean serum IgG ELISA responses are shown for each relevant Group to the 3D7 allele (A) $MSP1_{19}$ and (B) AMA1 antigens. (C) Individual and geomean responses are shown for each antigen and each relevant Group at the day 14, day 56 and dC-1 time-points. ** P < 0.01 by Mann-Whitney test (AMA1) and Kruskal-Wallis test with Dunn's Multiple Comparison test (MSP1). Group 5 and Groups not vaccinated with the antigen were excluded from the dC-1 analysis. (D) In vitro GIA of purified IgG was assessed at 10mg/mL. Individual data and medians are shown for each group at the dC-1 time-point. Pre-immunization (d0) sera were also pooled and the GIA tested for each of the four vaccinated groups. Responses >20% are generally regarded as positive.*

9.6 EFFICACY

All 6 un-vaccinated infectivity control volunteers were diagnosed with malaria. 1/9 volunteers (11%) in group 3 were sterilely protected (persistently PCR negative) and 2 demonstrated delay to diagnosis (diagnosis on day 13.0 & 13.5 post challenge). 1 volunteer in group 2 (11%) was not diagnosed until Day 18.0 post challenge. 1 volunteer in group 4 was not diagnosed until Day 15.0 post challenge. There was no significant difference in time to diagnosis between controls and any vaccinated group (Figure 12). There was no significant difference in parasite multiplication rates between vaccinees and controls (Figure 13). No volunteers underwent re-challenge.

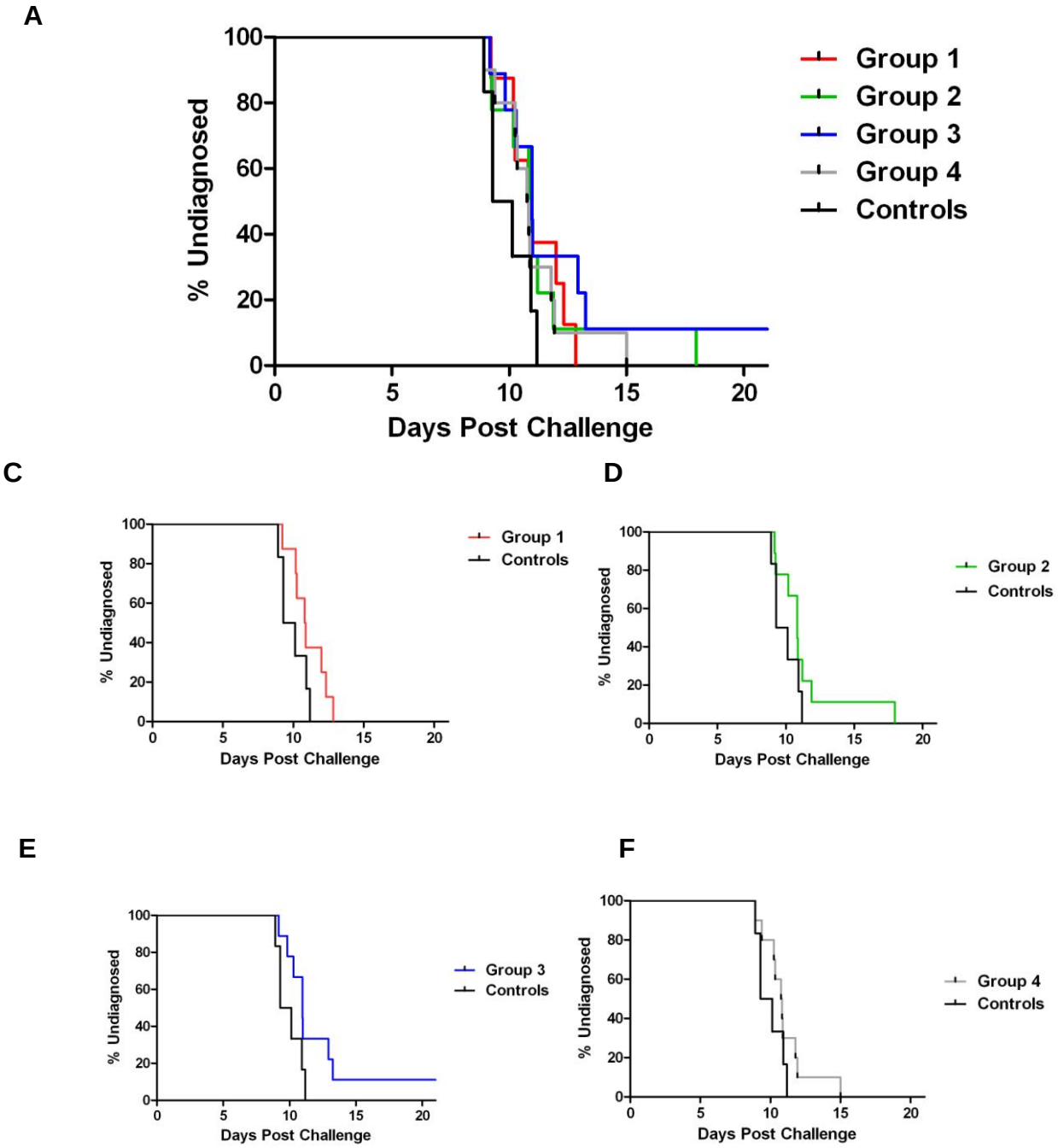


Figure 12: Kaplan-Meier survival analyses of time to patent parasitaemia in days (calculated from hours between mosquito bite and diagnosis) for vaccinees (n=36) versus unvaccinated controls (n=6). Figure 12A: All groups (P=0.13). Figure 12B Group 1; AdCh63-MVA MSP1 (P=0.13). Figure 12C: Group 2; AdCh63-MVA AMA1 (P=0.20). Figure 12D: Group 3; AdCh63-MVA MSP1+AMA1 (P=0.07). Figure 12E: Group 4; AdCh63-MVA MSP1+ME-TRAP (P=0.20).

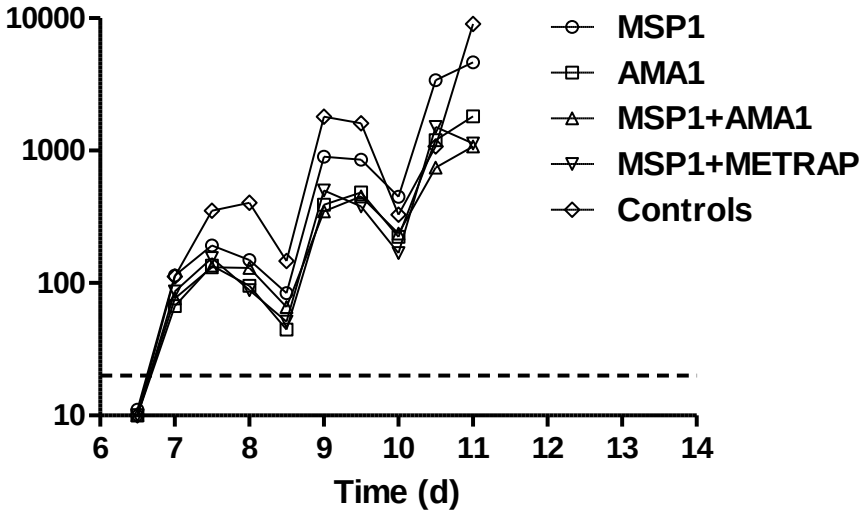


Figure 13: Geomean quantitative PCR data for each group post challenge. The lower limit of detection is indicated by the dotted line at 20 parasites/mL. MSP1= Group 1, AMA1 = Group 2, MSP1+AMA1 = Group 3, MSP1+ME-TRAP = Group 4, Controls = unvaccinated infectivity controls (group 5).

10. CONCLUSIONS & DISCUSSION

Heterologous prime-boost with AdCh63-MVA expressing the malaria antigens MSP1, AMA1 and ME-TRAP administered intramuscularly is safe and immunogenic in healthy malaria naive adults. Co-administration of AdCh63 and MVA vectored vaccines was also shown to safe, associated with an increased but still acceptable reactogenicity profile (consistent with increased vector dose administered).

AdCh63-MVA expressing the malaria antigens MSP1, AMA1 and ME-TRAP has been shown to be an extremely immunogenic regimen inducing not only strong T cell responses but substantial antibody responses. The median ELIspot response in group 1 is, to our knowledge, the highest yet reported following immunization with any subunit vaccine. In contrast to animal studies, co-administration of vaccines was associated with a reduction in the total T cell responses to each individual antigen when compared to single administration. AMA1 IgG responses were also significantly reduced when this vaccine was co-administered with the MSP1 vaccines. The reason for this is unclear. Further work will seek to understand this finding which has important implications for future sub-unit vaccines seeking to target multiple antigens.

Individuals in three groups (groups 2, 3 and 4) demonstrated a delay in time to diagnosis and one individual (group 3) was sterilely protected; the first time sterile protection has been demonstrated in any individual following vaccination with blood-stage malaria antigens alone. Given that parasite multiplication rates post challenge were identical for all groups, this partial efficacy is likely to reflect vaccine efficacy at the late liver-stage, where the 'blood-stage' antigens MSP1 and AMA1 are known to be expressed.

The study has also demonstrated that strong T cell responses (induced following AdCh63-MVA) against blood-stage antigens of *P. falciparum* are not associated with adverse outcome when the vaccinee is exposed to natural antigen, with no evidence of immunopathology. Moreover, these strong cellular responses did not appear to impact on acute blood-stage parasite growth rates – as widely observed in certain pre-clinical mouse malaria models. These data thus have important implications for future development of strong T cell inducing blood-stage malaria vaccines.

This AdCh63-MVA viral vectored vaccine regimen now provides a safe and clinically-relevant strategy for the development of vaccines against other difficult diseases where strong cellular and/or humoral immune responses are likely to be required for protection.